Onset of Dormancy in the Copepod
***Calanus pacificus californicus* off Southern California**

David M. Checkley, Jr.
Integrative Oceanography Division, 0218
Scripps Institution of Oceanography
La Jolla, CA 92093-0218
phone: (858) 534-4228 fax: (858) 822-0562 email: dcheckley@ucsd.edu

Award #: N00014-00-1-0172

LONG-TERM GOAL

Our long-range objectives are to understand and, as feasible, predict the distribution and abundance of pelagic organisms, particularly in regard to their physical environment and its changes. The objective of the proposed research is to identify the mechanism that controls onset of dormancy in open-ocean copepods and thus explain spatial and temporal variation in dormancy response. Specifically, we are developing a method that can be used to identify the conditions under which copepods prepare for dormancy in the field. This research will contribute to the following questions: (1) How does dormancy response affect population dynamics of open-ocean copepods? (2) What effect do short- and long-term environmental changes have on dormancy response of open-ocean copepods? (3) Do latitudinal differences in dormancy response of populations with broad ranges result from latitudinal differences in environmental stimuli alone (i.e. is population response to stimuli the same throughout the range)? (4) What controls the buildup and dispersal of deep aggregations of dormant copepods?

OBJECTIVES

(1) To develop a new, hormonal method for detecting preparation for dormancy in surface-dwelling, active *Calanus.*

(2) To observe preparation for dormancy in surface copepods in nature using this hormonal method; to compare the timing of preparation for dormancy with other indicators of onset of dormancy, including appearance and buildup of dormant *Calanus* at depth; and to compare timing of onset of dormancy with changes in environmental conditions that could induce dormancy.

(3) To determine whether aggregations of dormant *C. pacificus* are found in basins off southern California other than the Santa Barbara Basin and whether mid-depth layers of dormant *Calanus* are widespread in the Southern California Bight.

APPROACH

We are taking an integrated approach, including both field sampling and laboratory experiments, to examine onset of dormancy. We are developing a hormonal method using ecdysteroids, molting hormones, for detecting preparation for dormancy in *Calanus* 5^{th} copepodites (CVs). Onset of dormancy is being examined in a time series of field sampling in the San Diego Trough in the season when onset of dormancy occurs. This time series includes monitoring of multiple indicators of

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **30 SEP 2001**	2. REPORT TYPE	3. DATES COVERED **00-00-2001 to 00-00-2001**

4. TITLE AND SUBTITLE **Onset of Dormancy in the Copepod Calanus pacificus californicus off Southern California**	5a. CONTRACT NUMBER
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Integrative Oceanography Division, 0218, ,Scripps Institution of Oceanography,,La Jolla,,CA,92093**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR’S ACRONYM(S)
	11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

preparation for and onset of dormancy, including pooled ecdysteroid level, metabolic enzyme activity levels, and appearance and buildup of CVs at depth. Environmental conditions that could trigger onset of dormancy, including temperature, salinity, and phytoplankton concentration and taxonomic composition, are also monitored. We also examine broader patterns of spatial distribution of dormant *Calanus* off southern California to assess the relative importance of the dormant and active parts of the population and whether basins are sites of greater dormant *Calanus* abundance than elsewhere.

Ecdysteroid Method -- Ecdysteroid in many crustaceans and other arthropods is low during the postmolt and intermolt and high during premolt periods of the molt cycle. If this is so for copepods, then a shift in the molting stage distribution of CVs toward earlier molting stages, due to preparation for dormancy, would result in a decrease in population-level ecdysteroid. To control for environmental factors, such as temperature and food concentration, that could change the molting stage distribution and thus pooled ecdysteroid in *Calanus* CVs, we will compare change in pooled ecdysteroid in CVs to that in the previous developmental stage, the 4th copepodite (CIV), which should respond similarly to environmental conditions, but which does not become dormant off southern California. We expect the ratio of pooled ecdysteroid in CVs / pooled ecdysteroid in CIVs ("CV/CIV ecdysteroid ratio") to be constant over varying environmental conditions but to decrease when CVs are preparing for dormancy. Development of the ecdysteroid method is being conducted in three parts. First, we are characterizing ecdysteroid variation through the molt cycle in CVs and CIVs using a radioimmunoassay (RIA, method developed by Chang & O'Connor 1979) of individual copepods. Second, we are growing copepods to CIV and CV stages under several combinations of temperature and food and measuring pooled ecdysteroid under normal development. Finally, we are examining pooled ecdysteroid in field-collected copepods when preparation for dormancy or continuing development is inferred from changes in abundance of copepods at depth and jaw morphology.

San Diego Trough Time Series -- Repeated sampling in San Diego Trough (SDT) was conducted in 2000 over the season when onset of dormancy occurred. Vertically stratified zooplankton samples were collected and temperature, salinity, oxygen concentration, and fluorescence were measured using a multiple opening and closing net and environmental system (MOCNESS, Wiebe *et al.* 1985). Pigment and phytoplankton samples were collected from near the surface and at the chlorophyll maximum for determination of phytoplankton community composition. This time series provides a comprehensive dataset that is being used to examine changes in CV and CIV ecdysteroid in combination with other indicators of dormancy, particularly changes in CV distribution and abundance, and environmental factors, which may trigger onset of dormancy.

Distribution of Dormant *Calanus pacificus* Off Southern California -- Vertically stratified zooplankton samples were collected to depths up to 2000 m at five basin and five non-basin stations on cruises in January, August, and October 1999 and October 2000. *Calanus pacificus* CIV, CV, and adult females and males are being enumerated in samples collected on these cruises. Dormancy status of CVs is being estimated from developmental stage frequencies and citrate synthase (metabolic enzyme) activity of *Calanus* within the depth strata sampled.

This work comprises part of the PhD research of Ms. Catherine Johnson (clj@ucsd.edu)

WORK COMPLETED

Ecdysteroid titers were measured in individual *C. pacificus* CVs that had been grown to known times since molting at 16 °C. Ecdysteroid titers of individual, dormant *C. pacificus* CVs from nature were also measured. Tooth development stage distributions were determined for deep, dormant CVs, surface CVs at two dates when onset of dormancy was not occurring, and surface CVs at two dates when onset of dormancy was occurring, all at the San Diego Trough. Differences in pooled ecdysteroid in CVs were analyzed using a bootstrap simulation based on changes in molting stage distribution and ecdysteroid titer distributions at postmolt and non-postmolt molting stages.

Zooplankton sampling at the San Diego Trough was conducted on thirteen dates between April 2000 and March 2001. Enumeration of *Calanus* CIV, CV, and adult females and males has been completed for nine dates and partially completed for one other date.

Enumeration of *Calanus* CIV, CV, and adult females and males has been completed for three cruises, January and October 1999, and October 2000. Preliminary data analysis has been completed for the two October cruises.

RESULTS

Ecdysteroid Method -- Ecdysteroid titers were measured in individual *Calanus pacificus* CVs grown at 16 °C to different times during the molt cycle. The post- and intermolt (non-premolt) stages were about one third of the CV stage duration of four to five days. Ecdysteroid titer was variable throughout the molt cycle but, on average, was lowest 0 – 1 day after molting (mean = 18 pg), and highest 1 – 2 days after molting (mean = 47 pg), suggesting that the pattern of ecdysteroid variation in *Calanus pacificus* CVs may be similar to that in some crab megalopea, in which ecdysteroid titers are highest during the premolt stage but begin to increase in late intermolt (Spindler & Anger1986) The mean ecdysteroid titer of field-collected, dormant *C. pacificus* CVs from the San Diego Trough (mean = 7 pg) was lower than the lowest mean titer of laboratory-cultured CVs 0 – 1 day after molting.

The proposed ecdysteroid method assumes that during periods of preparation for dormancy, the stage frequency of tooth development of surface *C. pacificus* CVs should shift toward the postmolt stage. We tested this assumption by analyzing stage frequency distributions of tooth development for CVs and CIVs collected on San Diego Trough cruises when onset of dormancy was not (April, May 2000) and was (July, August 2000) occurring. These distributions were significantly different (heterogeneity χ^2, $p<0.001$) for the four dates analyzed, while CIV tooth development stage frequency distributions were not significantly different, suggesting that changes in the CV tooth stage distribution were not due to environmental changes. On the two dates when onset of dormancy was occurring, CV tooth development distribution was skewed toward postmolt stages compared to the earlier dates, as expected.

San Diego Trough Time Series -- The SDT time series documents the appearance and build up of CVs at depth during 2000. CVs begin to increase in abundance between 250 and 400 m in June (Fig. 1). CVs deeper than 200 m, presumed to be dormant, generally increase in abundance from June through October. CVs are least abundant at the surface in September and October. In November, CV abundance increases at the surface while abundance below 200 m decreases. Because buildup of CVs at depth is spread out over a long time period, onset of dormancy does not appear to be closely associated with particular values of the seasonal cues we measured. CVs first appear in deep water before the summer solstice and continue to increase in deep water until after the autumn equinox. In general, however, the onset of dormancy is associated with decreasing day length. Temperature in the upper 50 m, where most shallow CVs are found, increased from the beginning of sampling to a maximum in mid-August as CVs increased in abundance below 200 m. However, temperature in the upper 50 m decreased as one of the largest increases in CV abundance below 200 m occurred. The two largest increases in CV abundance below 200 m are during or immediately after peaks in subsurface chlorophyll concentration.

Distribution of Dormant *Calanus* Off Southern California -- The vertical distribution and abundance of dormant CVs at basin and non-basin stations off southern California were compared for October 1999 and October 2000. Vertical distribution patterns of deep CVs were variable across stations in both years. In general, the range of depths occupied by most deep *Calanus* CVs was broad, typically from about 200 m to 1400 m. The highest concentrations of dormant CVs were found in Santa Barbara Basin (SBB) in both years; however integrated abundance of dormant CVs in the SBB was lowest of the stations sampled in 1999 and second highest in 2000. The median, integrated abundance of deep CVs at basin stations was not significantly different from the median, integrated abundance at non-basin stations; however, the median, integrated abundance was different between 1999 and 2000 (Friedman two-way analysis with replicates; Johnson & Gendron, 2001).

IMPACT/APPLICATIONS

The temporal and spatial patterns of dormancy identified by this research contribute to understanding of the mechanisms controlling distribution and abundance of deep, dormant *C. pacificus* and will be critical to modeling *Calanus* population dynamics in this region. The distribution pattern of dormant *Calanus pacificus* CVs identified by this research suggests that all deep areas off southern California, not only basins, can act as sources for *Calanus pacificus* returning to the surface in spring. Our San Diego Trough field study is the first to resolve the timing of descent of *Calanus* CVs off southern California. In addition, this project provides evidence for strong interannual variability in the abundance of dormant *Calanus*.

Our molting hormone method will provide a means to examine the environmental conditions associated with preparation for dormancy by *Calanus* and other copepods in nature, off southern California and elsewhere. Identification of the stimuli that induce dormancy in *C. pacificus* will provide a basis for prediction of short-term interannual variability in the timing of onset of dormancy and thus formation of deep aggregations. The ecdysteroid method will also provide a means for identifying longer-term changes in the threshold stimuli that trigger dormancy, for example changes in response to global warming, in *Calanus* and other open-ocean copepod species.

TRANSITIONS

The ecdysteroid assay, in principle, should be able to be used by other investigators to detect preparation for dormancy in copepods. Our results should be useful to those modeling copepod dynamics and aggregation.

RELATED PROJECTS

This work follows prior, ONR-funded studies of diapausing *Calanus pacificus* in the Santa Barbara Basin (Osgood and Checkley 1997a,b). It also makes use of, and contributes to, the California Cooperative Fisheries Investigations, CalCOFI. Our work is also of interest to Drs. Carina Lange and Amy Weinheimer, of SIO, who study recent deposition in the Santa Barbara Basin. Copepod diapause is of interest to planktologists, fisheries oceanographers, acousticians, and biogeochemists worldwide.

REFERENCES

Chang, ES and JD O'Connor (1979) Arthropod molting hormones. In *Methods of Hormone Radioimmunoassay, 2nd ed.*, BM Jaffe and HR Behrman eds. pp. 797-814, Academic Press, New York.

Johnson, CL and JM Gendron (2001) Vertical distribution of dormant *Calanus pacificus* in basins and deep water off southern California. Poster presented at ASLO Aquatic Sciences Meeting, Albuquerque, NM.

Osgood, K.E., and D.M. Checkley, Jr. (1997a) Seasonal variations in a deep aggregation of Calanus pacificus in the Santa Barbara Basin. Mar. Ecol. Prog. Ser. 148:59-69.

Osgood, K.E., and D.M. Checkley, Jr. (1997b) Observations of a deep aggregation of Calanus pacificus in the Santa Barbara Basin. Limnol. Oceanogr. 42:997-1001.

Spindler, K-D and K Anger (1986) Ecdysteroid levels during the larval development of the spider crab *Hyas aranaeus.* General and Comparative Endocrinology, v. 64: 122-128.

Wiebe, PH, AW Morton, AM Bradley, RH Backus, JE Craddock, V Barber, TJ Cowles, and GR Flierl (1985) New developments in the MOCNESS, an apparatus for sampling zooplankton and micronekton. Marine Biology, v. 78: 313-323.

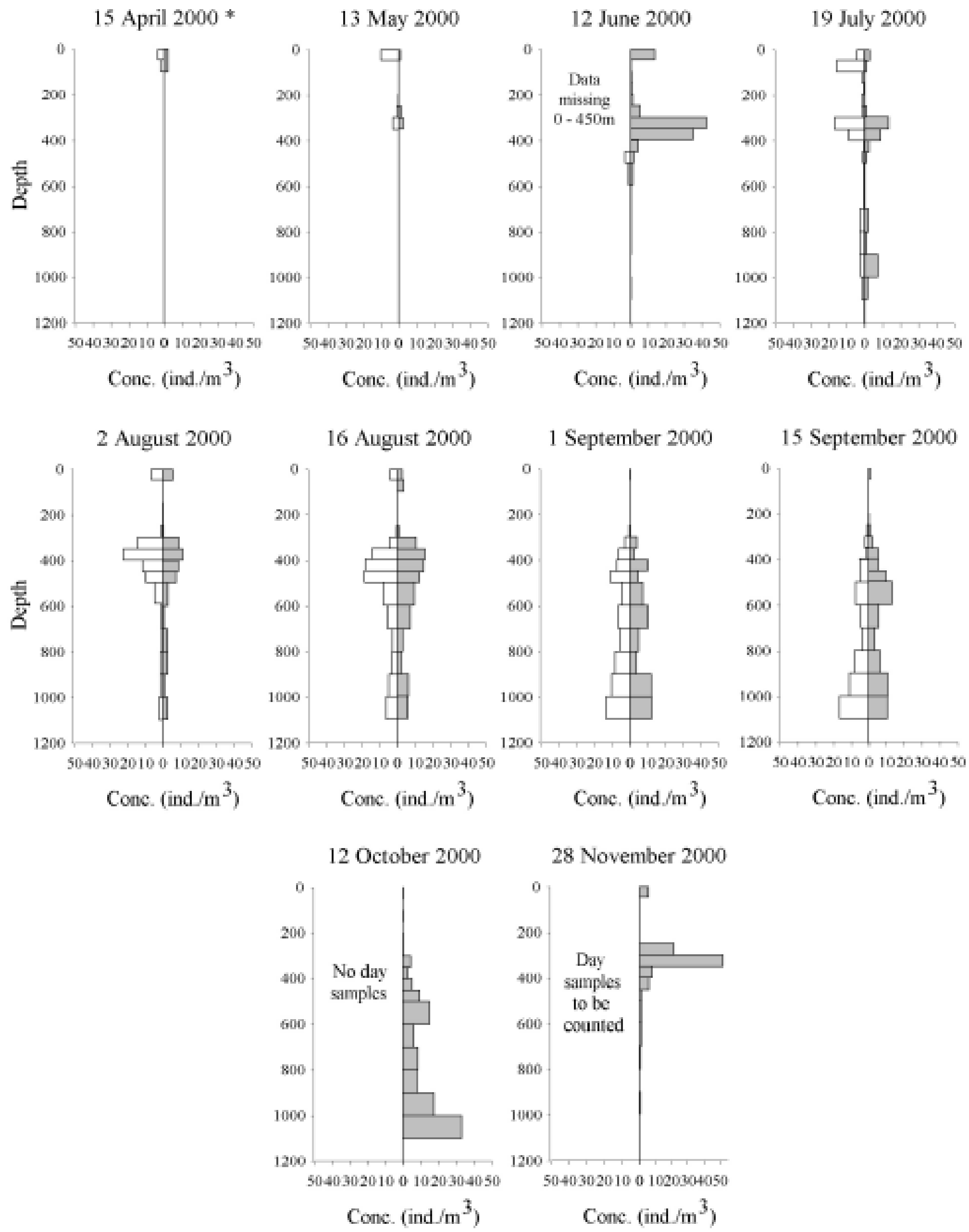

Figure 1: Vertical distribution of Calanus pacificus CVs in the San Diego Trough. White bars represent daytime distribution, and gray bars represent nighttime distribution. Samples collected 10/31/00, 1/18/01, and 3/1/01 remain to be counted. *Concentrations in April are uncorrected and may be in error by as much as 30 %.

[In April 2000, there are no CVs below 100 m. In May 2000 CV concentrations are less than 5 ind/m^3 between 250 and 350 m. From July 2000 to October 2000 deep CVs occupy the depth range from 250 to 1100 m. In November 2000 most deep CVs are between 250 and 450 m.]